SHORT REPORT

Detection of *Simkania negevensis* by culture, PCR, and serology in respiratory tract infection in Cornwall, UK

M G Friedman, S Kahane, B Dvoskin, J W Hartley

J Clin Pathol 2006;59:331–333. doi: 10.1136/jcp.2004.025601

Respiratory tract infections are often treated empirically without investigation to detect the aetiological agent, which may be a virus or a bacterium, including atypical pathogens such as *Chlamydia pneumoniae* or *Mycoplasma pneumoniae*. Recently, several types *Chlamydia*-like intracellular bacteria have been detected in environmental samples and clinical specimens. Little is known of their geographical distribution and potential pathogenicity. We describe the detection, by PCR and isolation in cell culture, of *Simkania negevensis* in nasopharyngeal aspirates of paediatric patients with bronchiolitis in Cornwall, UK. We also present serological evidence of exposure to the organism in 62% of adult patients and 46% of a sample of pregnant women. Empirical treatment of serious respiratory tract infection should consider the possible contribution of these organisms.

The aetiology of a large proportion of respiratory tract infections cannot be determined by standard diagnostic techniques. Even when advanced techniques are used, an aetiology may be found in only about 70% of adult upper and lower respiratory tract infections.¹ Novel *Chlamydia*-like microorganisms may play a role in such infections.^{2,3} Indeed, *Parachlamydia* may be involved as an agent of community acquired pneumonia⁴ and inhalation pneumonia,⁵ while *Simkania negevensis*^{6,7} has been associated with bronchiolitis in infants in Israel⁸ and in Canada⁹ and with community acquired pneumonia in adults in Israel.¹⁰ A high seroprevalence of antibodies to this penicillin resistant microorganism has been found in various population groups, ranging from 39% of 42 healthy adults in Brooklyn, USA, to 68% of a sample of 100 pregnant women in Vancouver, Canada and 68% of 106 blood donors in Aarhus, Denmark.²

In this study, we report the prevalence of antibodies to the organism in 200 healthy adults and 120 patients with various respiratory tract infections in Cornwall, UK. We also report its detection by PCR in 100 nasopharyngeal aspirates (NPA) of children with bronchiolitis (n = 222), and its isolation in cell culture from 70 aspirates.

MATERIALS AND METHODS

Serum samples were obtained from 200 presumably healthy pregnant women (aged 16–42 years) (healthy adult control sample). Approval for anonymous testing was given by the ethics committees of Cornwall the Public Health Laboratory Service. Sera were also obtained from 120 patients with evidence of respiratory tract infection (aged 1–55 years). All sera were stored at –20°C until testing. Nasopharyngeal specimens were also taken from children sufficiently ill to be admitted to hospital with cough and wheeze and who had diagnosed with bronchiolitis, and submitted for analysis. The samples were stored at –70°C until testing. Detection of IgG

and IgA antibodies to *S. negevensis* was by a previously described ELISA technique.¹¹

DNA for PCR studies was prepared from NPA samples by the QIAamp blood kit (Qiagen, Hilden, Germany) according to the manufacturer's directions and amplified by primers ZpF and ZpR as previously described.⁸ In some experiments, *S. negevensis* specific sequences were also amplified by nested PCR in which primers ccF and ccR, recognising conserved chlamydial 16S ribosomal DNA sequences were used for the outer set and ZpF and ZpR for the inner set. An additional nested PCR was carried out for amplification of another segment of the *S. negevensis* genome using primers AF and BR and IntF and IntR. The outer primers amplify a 1099 bp fragment of the 23S rDNA, while the inner ones amplify a 338 bp fragment of the *S. negevensis* intron.¹² The two nested PCRs have been described previously.¹³ Negative controls without DNA were included in each assay and were also processed through the second round of amplification. Negative control results other than negative would invalidate all PCR results.

Protocols for isolation of *Simkania* and *Simkania*-like microorganisms from NPA were performed as described previously.⁸

RESULTS AND DISCUSSION

The prevalence of IgG antibodies specific for *S. negevensis* was 46% in the sample of healthy adult controls. In the patients with respiratory tract infection, as shown in fig 1, IgG antibodies were increasingly prevalent with increasing age, starting from 15% in children aged 1–4 years and increasing to 62% in 29 adults aged 16–55 years ($p < 0.001$, by χ^2 test for linear trend in proportions; EpiInfo, version 6). By age range, the pregnant women appeared to be younger than the adult patients. This does not explain the lower seropositivity rate, as they were actually older. While 72% of the patients (21/29) were in the age range of 16–20 years, only 12.5% of the controls were in that age range; only two patients (1%) were older than 35 years, compared with 22 controls (11%). The seropositivity rate to *S. negevensis* (62%) was higher in the adult patients than in the healthy controls (46%); however, the difference, with or without adjustment for age, was not statistically significant. The seropositivity rates found in both groups of adults are consistent with figures previously published for adults in this and other parts of the world.^{2,11}

S. negevensis specific IgA antibodies were found in 6 of the 200 healthy women (3%), and in 5 of the 29 adult patients aged 16–55 years (17%), which is a statistically significant difference ($p = 0.004$, 95% confidence interval 0.3% to 28%). Organism specific IgA may be an indication of current or recent infection, and elevated or rising *S. negevensis* IgA titres have been described in patients with community acquired pneumonia in the Negev.¹⁰ Convalescent serum samples were not available from the patients with respiratory tract

Abbreviation: NPA, nasopharyngeal aspirates

Figure 1 *S. negevensis* IgG and IgA antibody seroprevalence in 120 patients with respiratory tract infection, by age group.

infection in this study, which precluded the possibility of determining aetiology on the basis of changing titres of specific antibody.

With respect to possible crossreactivity with other Chlamydiae or Chlamydia-like microorganisms, we have shown that there is no crossreactivity with *C. pneumoniae* antigens in the ELISA assay we used,¹¹ and Yamaguchi *et al* also found no such crossreactivity in the microimmunofluorescence test that they developed to test for antibodies to *S. negevensis*, compared with *C. pneumoniae*.¹⁴ The situation with regard to *Parachlamydia* species is less clear as few serological studies have been published with respect to these agents. This will be important to investigate, as Greub *et al* have shown some serological evidence that Parachlamydiaceae may be agents of pneumonia in poly-traumatised intensive care patients.⁵

S. negevensis was detected by both PCR and isolation in 27% of 222 NP samples, by one of these methods in 23% of the samples, and by neither method in 50% of the samples. Eight isolates were grown in quantity for further characterisation. Table 1 shows that PCR was able to detect more positive samples than culture under the conditions used, while 84% of culture positive samples were PCR positive. This suggests that PCR is more sensitive than culture.

The PCR results shown in table 1 were obtained using the PCR originally described for detection of *S. negevensis* DNA.⁸ Later, 65 of the isolation positive samples were tested again using nested primers for 16S rDNA, of which 59 were found to be positive. All except three of these samples showed the presence of an intron (characteristic of the type strain of *S. negevensis*)¹² in the 23S rDNA, when tested by the nested PCR specific for this intron, thus confirming the identity of the organism. While the three exceptions may indicate the presence of a different type of *Simkania*-like microorganism, it is also possible that the sensitivity of the 16S rDNA PCR was higher than that of the intron (23S rDNA) PCR assay. In that case, some DNA samples (containing less DNA) might be

Table 1 Correlation of isolation positivity and PCR positivity for 222 NPA samples

	PCR+	PCR–	Total
Isolation +	59	11	70
Isolation –	41	111	152
Total	100	122	222

amplified with one set of primers and not the other. The fact that we found no samples that amplified with the intron primers but not with the 16S primers supports this possibility.

In the present study, of eight children who had an additional NP sample taken within 7 days of the first sample, the results of the two samples were the same in seven cases. In four cases, the results were both negative and in three the results were both positive. In one case the first sample was positive by PCR and the second, taken 5 days later, was negative. Thus, where additional NP specimens were available for investigation, the results of the isolation and PCR techniques were consistent between specimens.

Respiratory tract infections are a major health burden throughout the world. In studies using optimum laboratory technology, an aetiological agent can be found in only 50–70% of cases. We report here that past infection with *S. negevensis* or closely related microorganisms is prevalent in Cornwall, UK, with prevalence increasing with age. More extensive studies are needed to confirm the possible association of *S. negevensis* with respiratory tract infection in Cornwall, and molecular studies are needed to determine the degree of similarity of the UK clinical isolates to those obtained in Israel and other parts of the world. Such studies are now underway in our laboratories. If this organism or other recently described environmental chlamydiae¹⁵ contribute significantly to respiratory illness in the UK and elsewhere, new diagnostic tests will require development, and standard treatment modalities may need adjustment.

ACKNOWLEDGEMENTS

We thank N. Givon for her assistance with statistical analyses.

Authors' affiliations

M G Friedman, S Kahane, B Dvoskin, Department of Virology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel

J W Hartley, Department of Clinical Microbiology, Royal Cornwall Hospital, Triliske, Truro, Cornwall, UK

Statement of competing interests: The authors of this manuscript have no competing interests.

Correspondence to: Dr M G Friedman, Department of Virology, Faculty of Health Sciences, Ben Gurion University of the Negev, PO Box 653, Beer Sheva, Israel 84105-IL; maureen@bgumail.bgu.ac.il

Accepted for publication 13 April 2005

TAKE HOME MESSAGES

- Several types of *Chlamydia*-like intracellular bacteria have been detected in environmental samples and clinical specimens over recent years, but little is known of their geographical distribution and potential pathogenicity.
- We found evidence of *Simkania negevensis* in nasopharyngeal aspirates of paediatric patients with bronchiolitis.
- Evidence of exposure to *S. negevensis* was found in % of 200 pregnant healthy women (controls) and 62% of adult patients with respiratory tract infection. Seropositivity appears to increase with increasing age.
- This organism should be considered a possible causative factor in serious respiratory tract infection.

REFERENCES

- 1 Lieberman D, Lieberman D, Korsonsky I, *et al.* A comparative study of the etiology of adult upper and lower respiratory infections in the community. *Diagn Microbiol Infect Dis* 2002;**42**:21–8.
- 2 Friedman MG, Dvoskin B, Kahane S. Infections with the Chlamydia-like microorganism Simkania negevensis, a possible emerging pathogen. *Microbes Infect* 2003;**5**:1013–21.
- 3 Greub G, Raoult D. Parachlamydiaceae: potential emerging pathogens. *Emerg Infect Dis* 2002;**148**:625–30.
- 4 Marrie TJ, Raoult D, La Scola B, *et al.* Legionella-like and other amoebal pathogens as agents of community-acquired pneumonia. *Emerg Infect Dis* 2001;**7**:1026–9.
- 5 Greub G, Boyadjiev I, La Scola B, *et al.* Serological hint suggesting that Parachlamydiaceae are agents of pneumonia in polytraumatized intensive care patients. *Ann N Y Acad Sci* 2003;**990**:311–19.
- 6 Everett KD, Bush RM, Andersen AA. Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. *Int J Syst Bacteriol* 1999;**49**:415–40.
- 7 Kahane S, Everett KD, Kimmel N, *et al.* Simkania negevensis strain ZT: growth, antigenic and genome characteristics. *Int J Syst Bacteriol* 1999;**49**:815–20.
- 8 Kahane S, Greenberg D, Friedman MG, *et al.* High prevalence of “Simkania Z”, a novel Chlamydia-like bacterium, in infants with acute bronchiolitis. *J Infect Dis* 1998;**177**:1425–9.
- 9 Greenberg D, Banerji A, Friedman MG, *et al.* High rate of Simkania negevensis among Canadian Inuit infants hospitalized with lower respiratory tract infections. *Scand J Infect Dis* 2003;**35**:506–8.
- 10 Lieberman D, Kahane S, Lieberman D, *et al.* Pneumonia with serological evidence of acute infection with the Chlamydia-like microorganism “Z”. *Amer J Respir Crit Care Med*, 1997;578–82.
- 11 Friedman MG, Galil A, Greenberg S, *et al.* Seroprevalence of IgG antibodies to the chlamydia-like microorganism ‘Simkania Z’ by ELISA. *Epidemiol Infect* 1999;**122**:117–23.
- 12 Everett KD, Kahane S, Bush RM, *et al.* An unspliced group I intron in 23S rRNA links Chlamydiales, chloroplasts and mitochondria. *J Bacteriol* 1999;**181**:4734–40.
- 13 Kahane S, Platzner N, Dvoskin B, *et al.* Evidence for the presence of Simkania negevensis in drinking water and reclaimed waste water in Israel. *Appl Environ Microbiol* 2004;**70**:3346–51.
- 14 Yamaguchi T, Yamazaki T, Inoue M, *et al.* Prevalence of antibodies against Simkania negevensis in a healthy Japanese population determined by the microimmunofluorescence test. *FEMS Immuno Med Microbiol* 2005;**43**:21–7.
- 15 Corsaro D, Venditti D, Valassina M. New parachlamydial 16S rDNA phylotypes detected in human clinical samples *Res Microbiol* 2002;**153**:563–7.